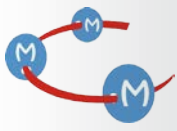


Quantitative image based analysis of endocrine disruptor effects on mitochondria morphology-function in prostate cancer cells.



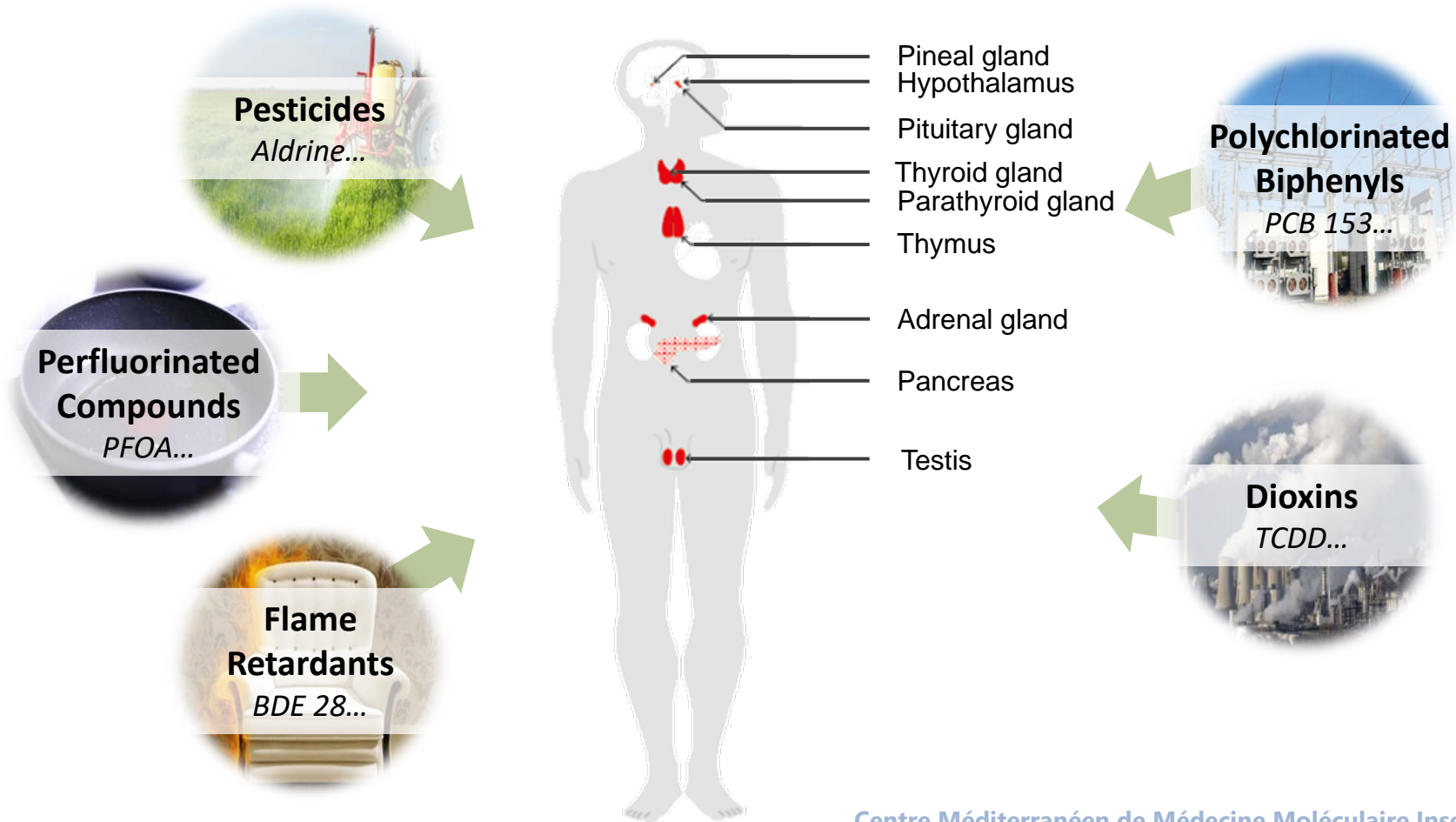
Charazac Aurélie, Decondé le Butor Célia, Giulietti Kévin, Lobaccaro Jean Marc, Baron Silvère, Gilleron Jérôme, Fénichel Patrick, Descombes Xavier, Bost Frédéric, Clavel Stéphan & Chevalier Nicolas.

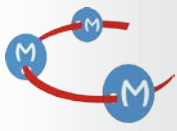


Endocrine Disruptor Compounds


« An endocrine disruptor is an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub) populations. »

World Health Organization





Major challenge

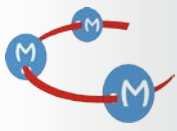
	SIN LIST	DANISH EPA	TEDX	EU REACH SVHC
Acceptation of WHO definition	✓	—	✗	✓
Based on risk assessment	✗	✗	✗	✗
List updating	✗	—	✓	✓

Adapted from EndocrineScience.org



Registration, **E**valuation and **A**utorization of **C**hemicals

└→ Promotes alternative methods for the hazard assessment of substances.



Major challenge

	SIN LIST	DANISH EPA	TEDX	EU REACH SVHC
Acceptation of WHO definition	✓	—	✗	✓
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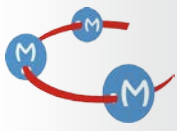


Registration, **E**valuation and **A**utorization of **C**hemicals

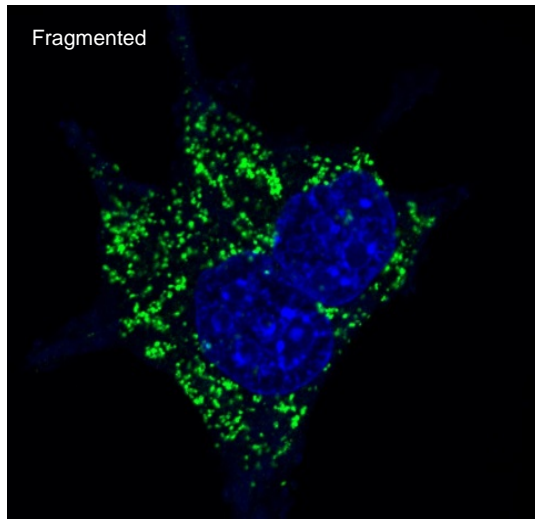
└ Promotes alternative methods for the hazard assessment of substances.

Urgent need for multiparametric, robust and high throughput cell-based assay :

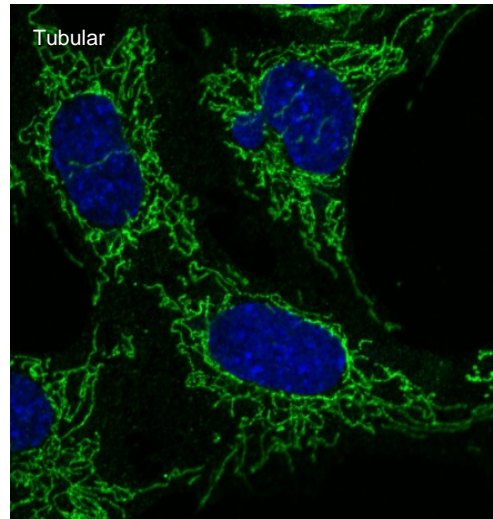
- ➔ To investigate the mechanisms underlying the adverse effects of known EDCs
- ➔ To identify new compounds with endocrine-disrupting potential



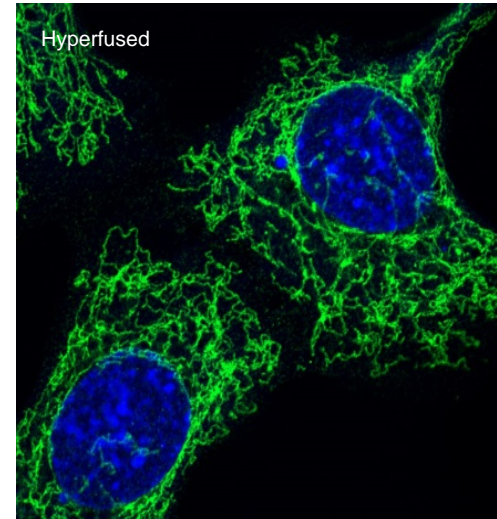
Mitochondria, a cell sensor



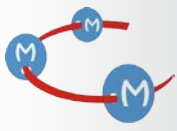
Apoptosis



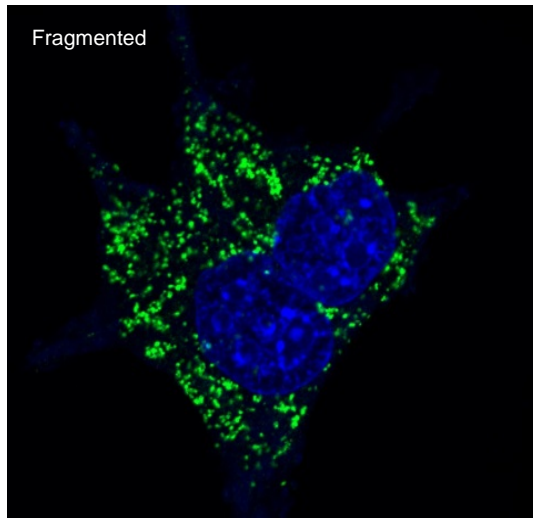
Healthy



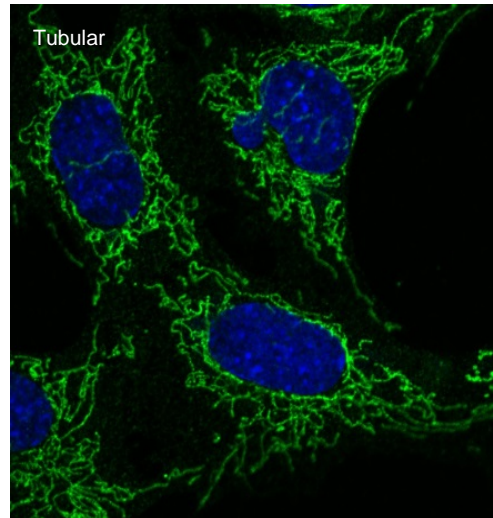
Stress



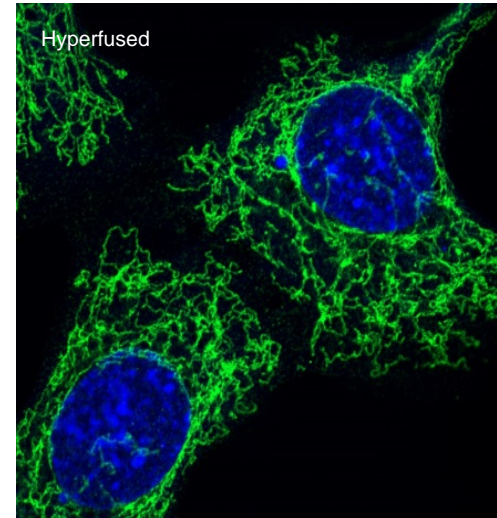
Mitochondria, a cell sensor



Apoptosis



Healthy



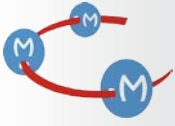
Stress

Calcium signalling

Cell growth and
differentiation

Cell cycle control

Cell death



Mitochondria, a cell sensor

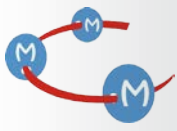
Alteration of **mitochondrial forms and functions** by EDCs ?

1) Quantitative image based analysis of mitochondrial functions

→ High throughput screening

2) Computational image based analysis of mitochondrial morphology

→ Image analysis and classification



Multiparametric cell-based assay

Automated image acquisition

(Nikon A1R-20X)

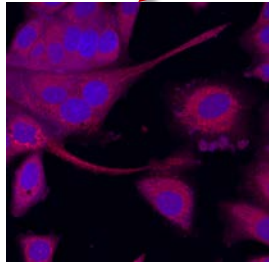
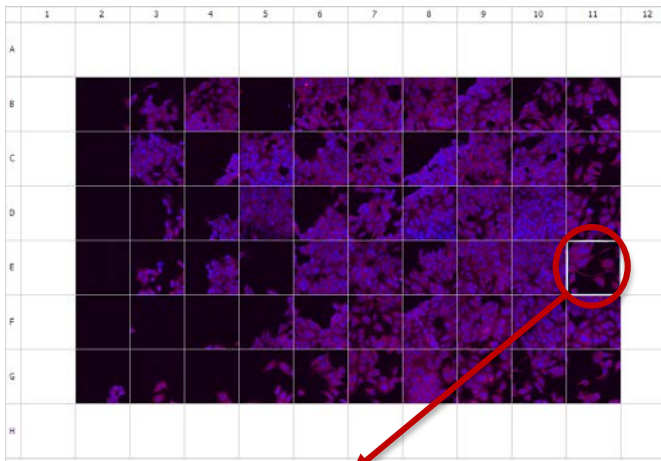
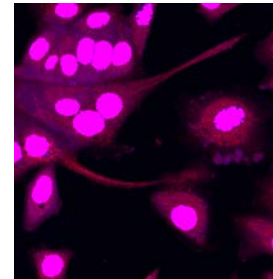
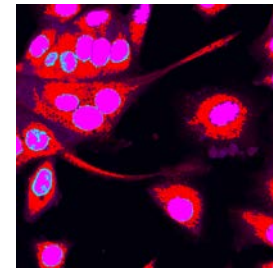


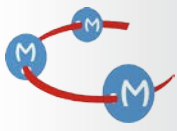
Image processing



→ 1) **Dapi masking :**
cell counting



→ 2) **Dye masking :**
fluorescence intensity



Multiparametric cell-based assay

Automated image acquisition

(Nikon A1R-20X)

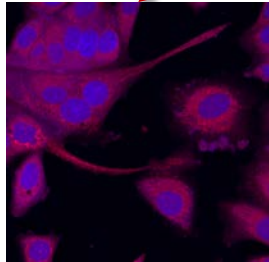
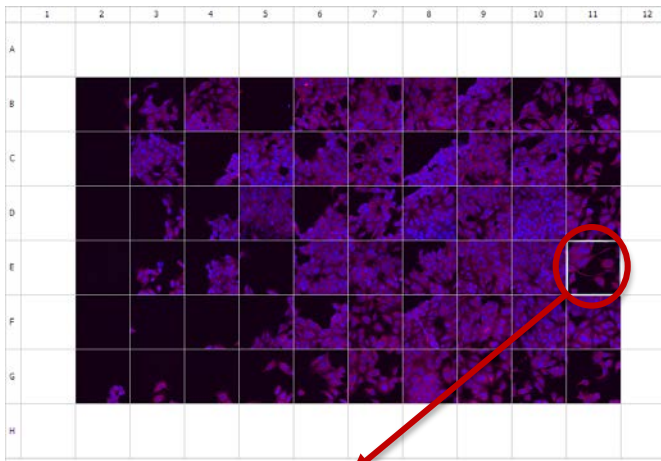
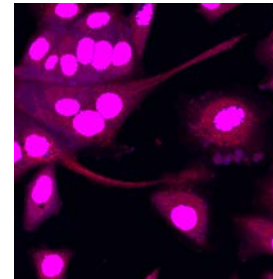
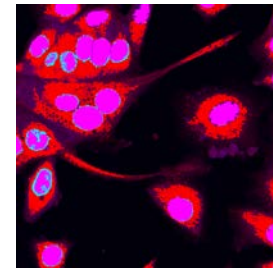


Image processing

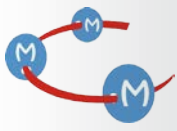


→ 1) **Dapi masking :**
cell counting



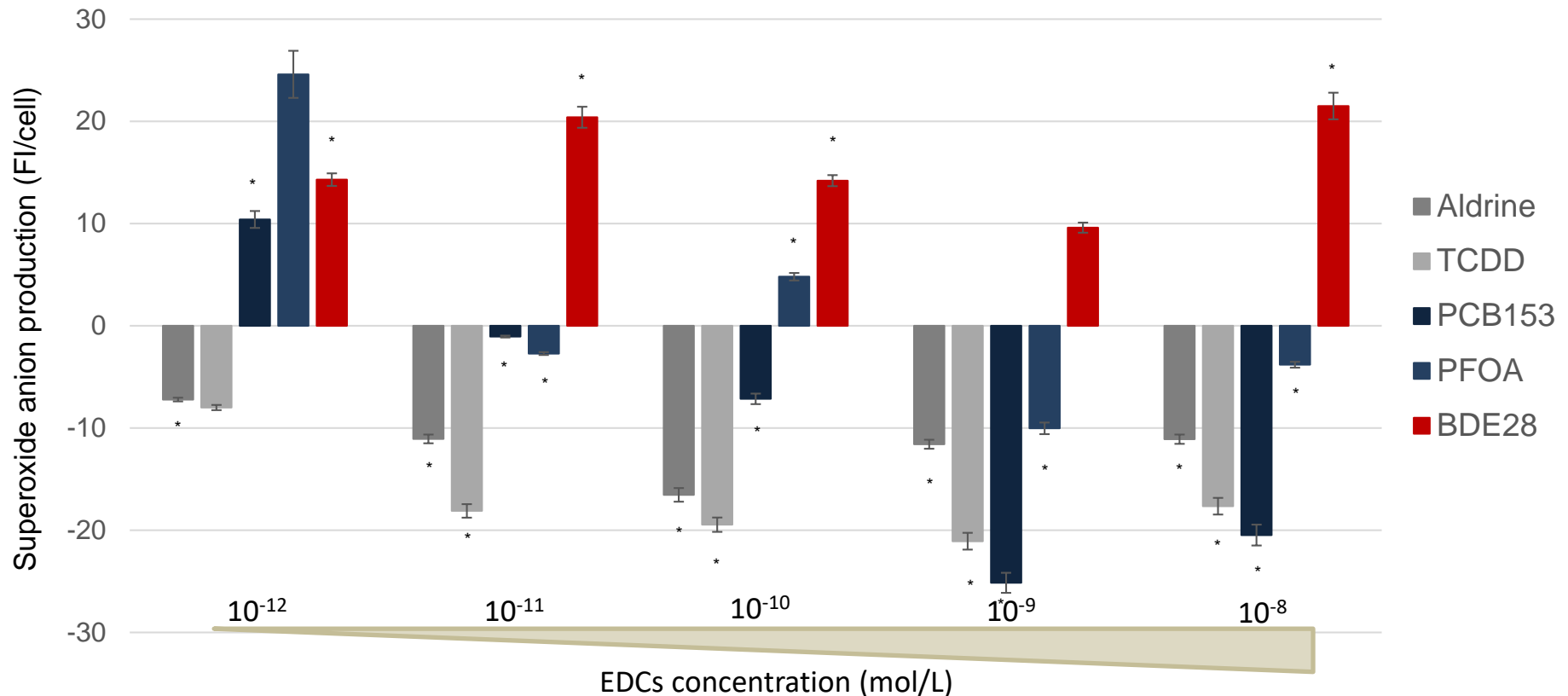
→ 2) **Dye masking :**
fluorescence intensity

- **Multiparameter :**
 - mitochondrial membrane potential (MitoTracker Red™)
 - superoxide anion production (MitoSox™)
 - mitophagy (Keima probe)

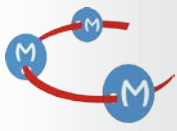


EDCs affect the mitochondrial function

- ROS production on DU145:

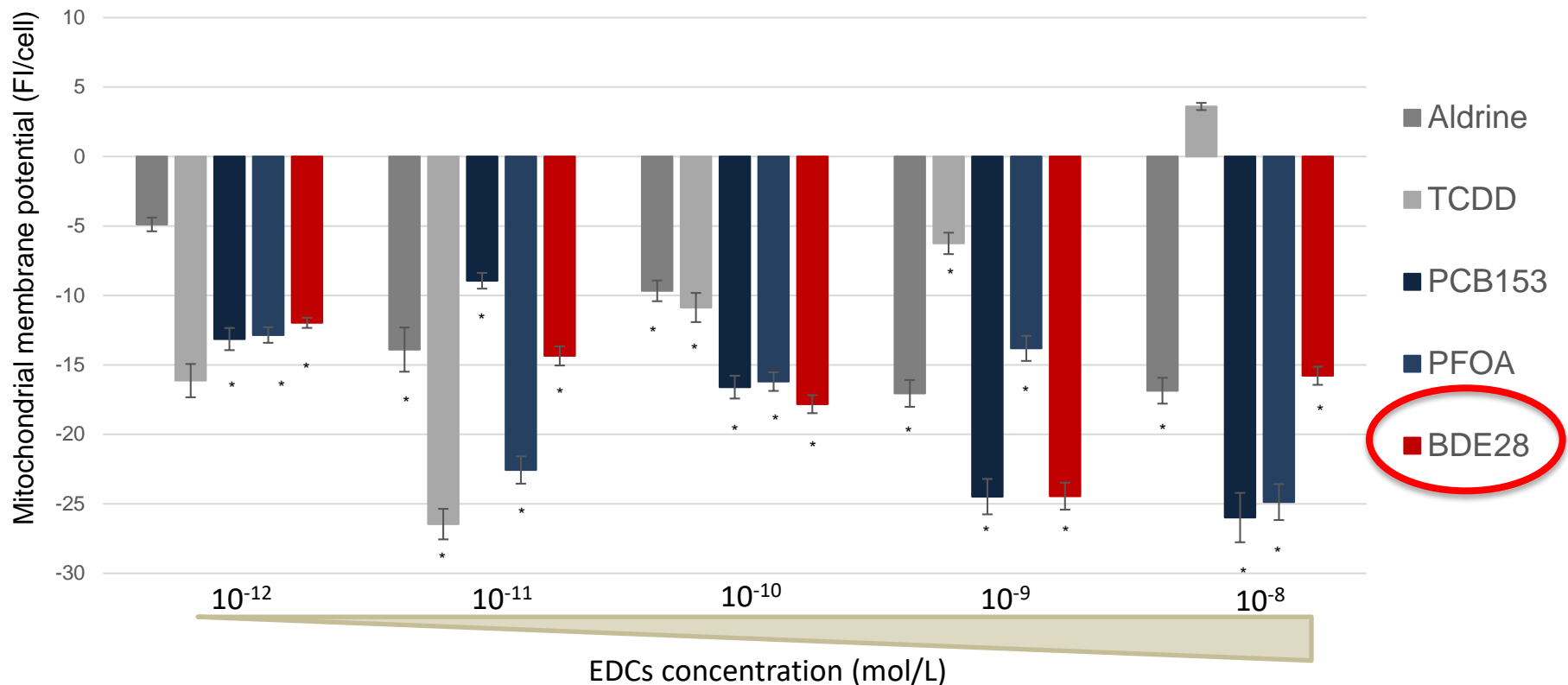


* significantly different from the control, $p \leq 0.005$.

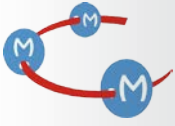


EDCs affect the mitochondrial function

- Mitochondrial membrane potential on DU145 :

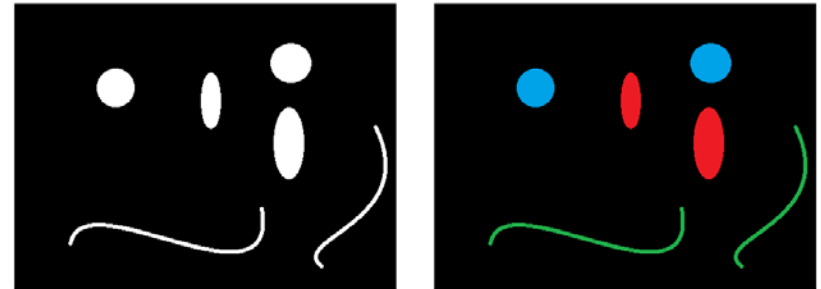
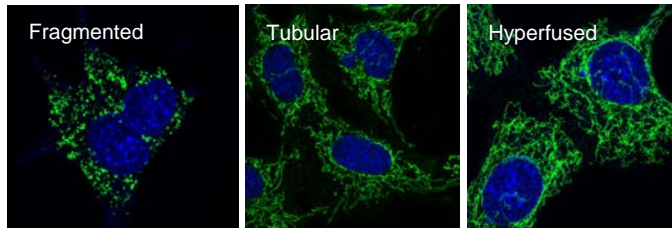


* significantly different from the control, $p \leq 0.005$.

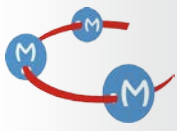


Forms classification of mitochondrial network

- Collaboration with

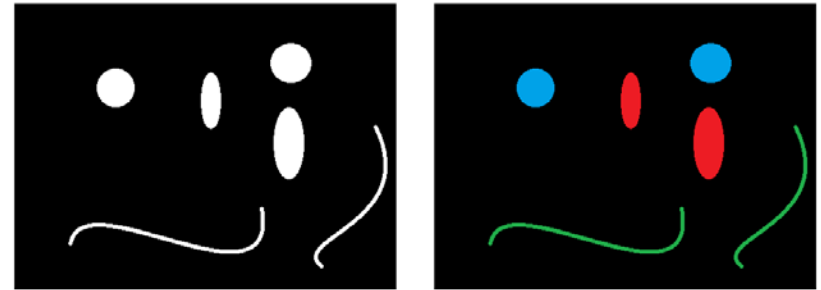
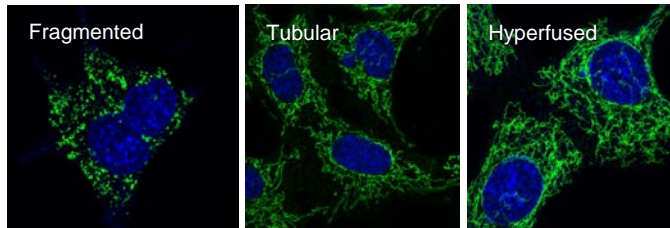


Fragmented, Tubular, Hyperfused



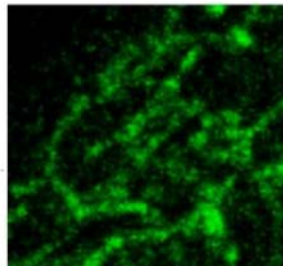
Forms classification of mitochondrial network

- Collaboration with

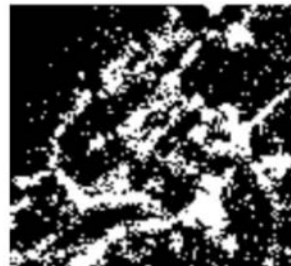


Fragmented, Tubular, Hyperfused

Image

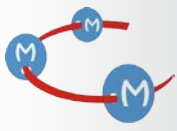


Basic
thresholding
method



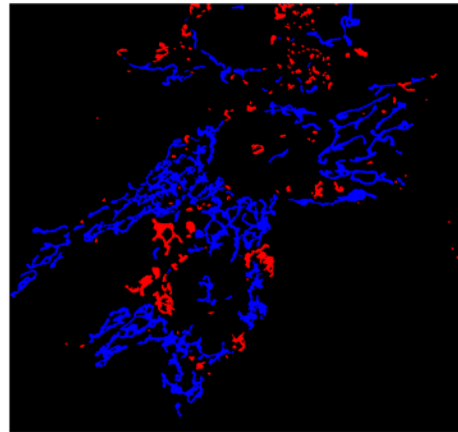
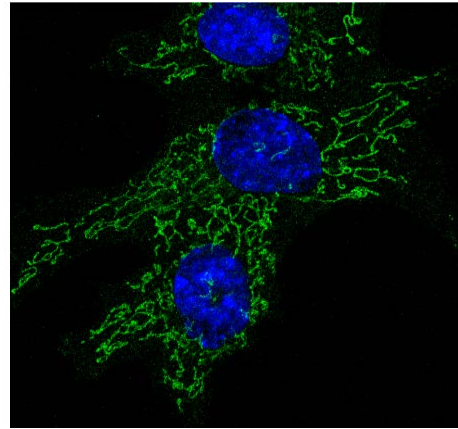
Developed
method

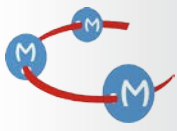




Exemple of developed method

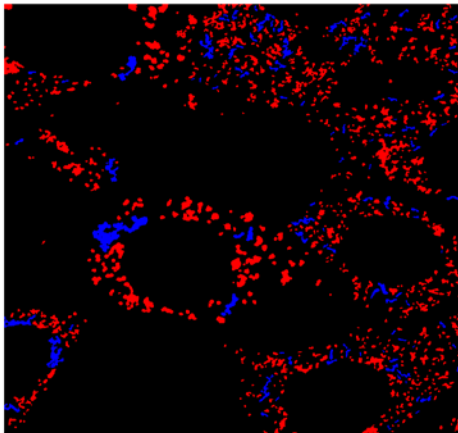
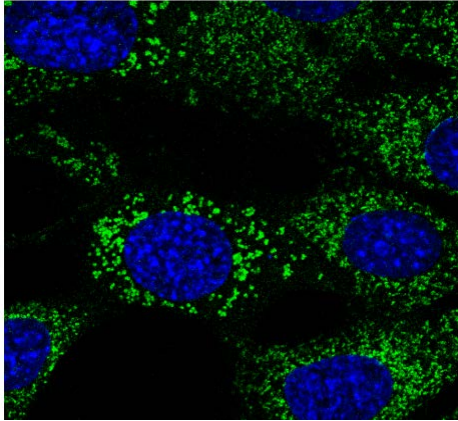
Tubular



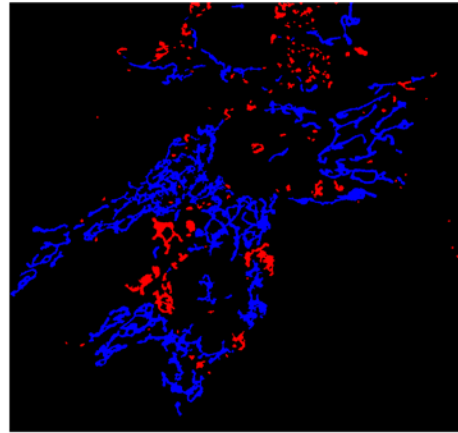
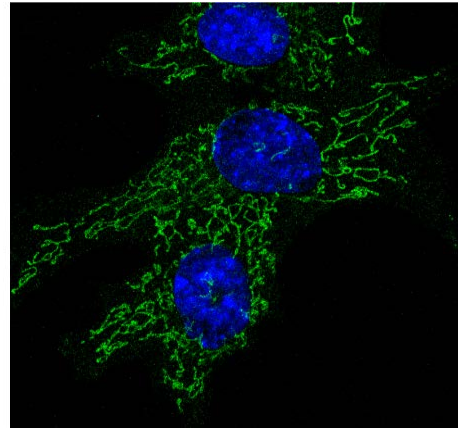


Exemple of developed method

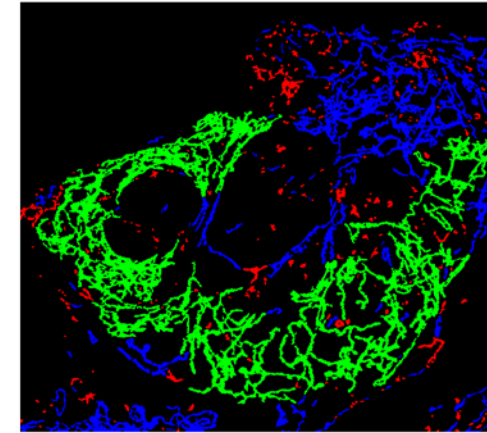
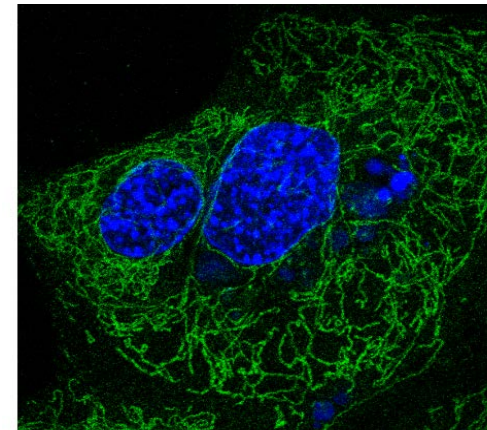
Fragmented
(Rotenone, 100nM)

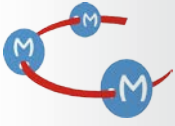


Tubular



Fused
(Compound C, 20μM)



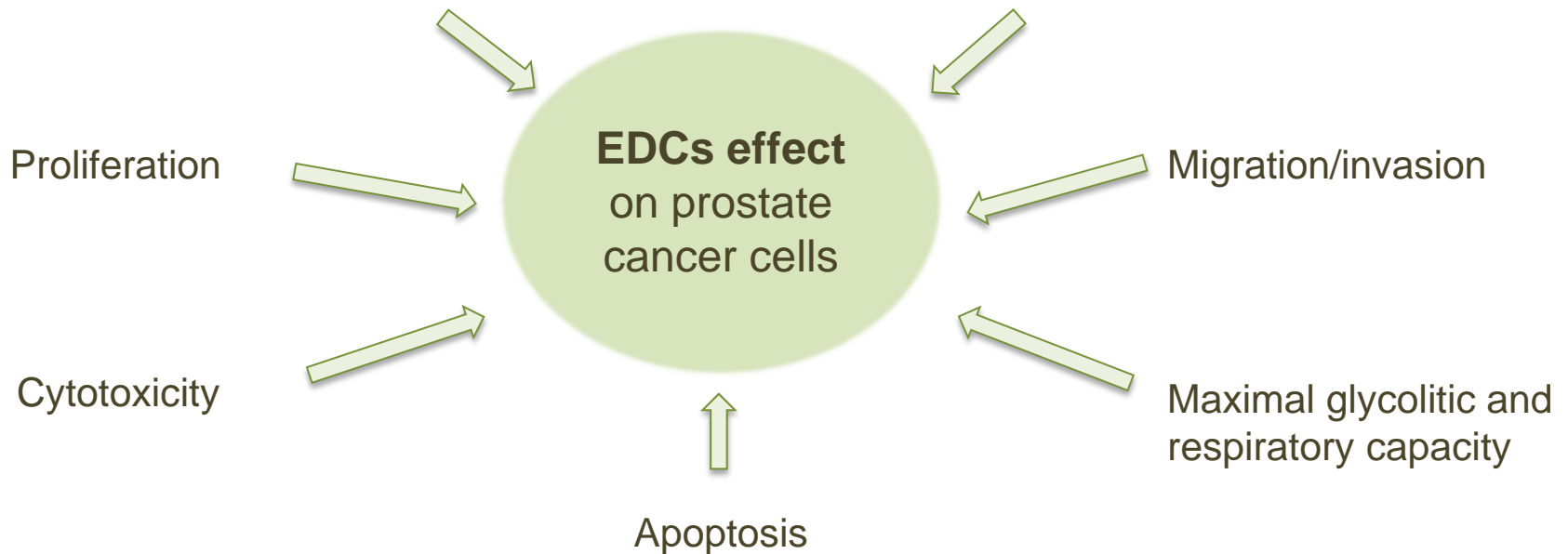


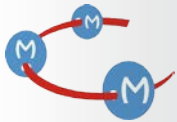
Final Goal

- Development of a multiparametric high throughput cell based screening :

Mitochondrial functional parameters

Mitochondrial morphological parameters



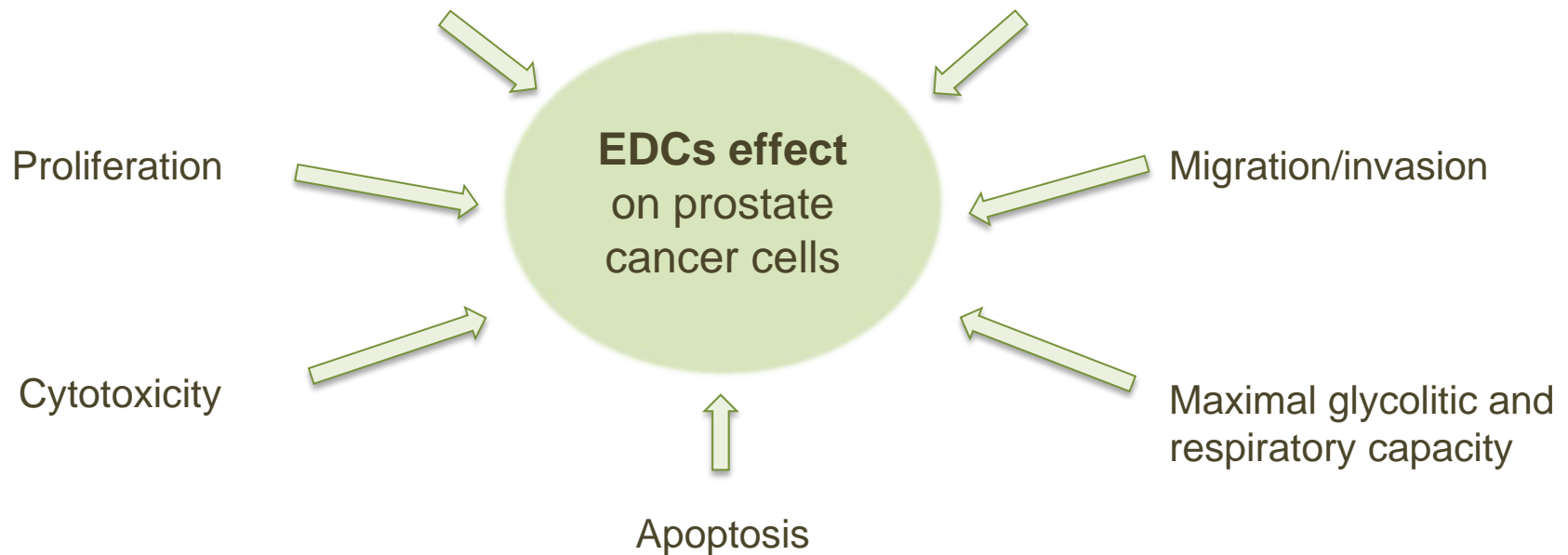


Final Goal

- Development of a multiparametric high throughput cell based screening :

Mitochondrial functional parameters

Mitochondrial morphological parameters



- ➡ Signature of EDCs based on their effects on cell metabolism
- ➡ New perspective in identification and characterization of EDCs



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Faustine Pastor

